Minireview

Control of glycogen deposition

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Abstract Traditionally, glycogen synthase (GS) has been considered to catalyze the key step of glycogen synthesis and to exercise most of the control over this metabolic pathway. However, recent advances have shown that other factors must be considered. Moreover, the control of glycogen deposition does not follow identical mechanisms in muscle and liver. Glucose must be phosphorylated to promote activation of GS. Glucose-6-phosphate (Glc-6-P) binds to GS, causing the allosteric activation of the enzyme probably through a conformational rearrangement that simultaneously converts it into a better substrate for protein phosphatases, which can then lead to the covalent activation of GS. The potency of Glc-6-P for activation of liver GS is determined by its source, since Glc-6-P arising from the catalytic action of glucokinase (GK) is much more effective in mediating the activation of the enzyme than the same metabolite produced by hexokinase I (HK I). As a result, hepatic glycogen deposition from glucose is subject to a system of control in which the 'controller', GS, is in turn controlled by GK. In contrast, in skeletal muscle, the control of glycogen synthesis is shared between glucose transport and GS. The characteristics of the two pairs of isoenzymes, liver GS/GK and muscle GS/HK I, and the relationships that they establish are tailored to suit specific metabolic roles of the tissues in which they are expressed. The key enzymes in glycogen metabolism change their intracellular localization in response to glucose. The changes in the intracellular distribution of liver GS and GK triggered by glucose correlate with stimulation of glycogen synthesis. The translocation of GS, which constitutes an additional mechanism of control, causes the orderly deposition of hepatic glycogen and probably represents a functional advantage in the metabolism of the polysaccharide.

Key words: Glycogen; Glycogen synthase; Liver; Muscle; Control; Glucose-6-phosphate

1. Introduction

Glycogen accumulation is a physiological response in mammals to the increase in blood glucose concentration that occurs after a meal. The biochemical pathway that allows the incorporation of new glucose units into a growing glycogen molecule is fairly well known and involves the successive action of several proteins and enzymes. Glycogen synthase (GS) catalyzes the addition of glucose residues to the non-reducing end of a nascent glycogen chain through α-1,4-glycosidic bonds using UDP-glucose (UDP-Glc) as a substrate. Traditionally, this enzyme, which catalyzes the key step of glycogen synthesis, has been considered to exercise most of the control over this metabolic pathway. In fact, GS activity is highly regulated through phosphorylation at multiple sites and allosteric effectors [1], mainly glucose-6-phosphate (Glc-6-P) [2] and, furthermore, the reaction catalyzed by GS is rate limiting for glycogen synthesis in all organs [3]. However, recent advances have shown that the control of glycogen deposition does not exclusively reside in GS and other factors must be considered. Moreover, as discussed below, the control of glycogen deposition does not follow identical mechanisms in muscle and liver (Fig. 1).

2. Differences between liver and muscle glycogen metabolism

First, let us point out the main differences in how glucose is handled in liver and muscle. In the hepatocyte, extracellular and intracellular glucose are in equilibrium due to the high capacity of the main hepatic glucose transporter GLUT-2 [4]. Glucokinase (GK; hexokinase IV (HK IV) is the predominant glucose-phosphorylating enzyme in hepatocytes (and in insulin- and glucagon-secreting cells of the pancreas) [5,6]. GK is a monomeric 50 kDa protein that exhibits kinetic properties quite distinct from those of the other members of the mammalian HK family. It is not inhibited by its product, Glc-6-P, and it has a sigmoidal saturation curve and a relatively low affinity for glucose (Hill coefficient = 1.5–1.8, $S_{0.5}$ = 2–8 mM, depending on the species), so that the flux through GK is sensitive to fluctuations in the concentration of its substrate in the physiological range [5]. In the liver, the activity of GK is acutely modulated by a GK regulatory protein (GKRP) that binds and inhibits GK competitively with respect to glucose. The inhibitory effect of GKRP is enhanced by fructose 6-phosphate and suppressed by fructose 1-phosphate, both of which bind to GKRP and modify its affinity for GK [7].

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Abbreviations: GS, glycogen synthase; Glc-6-P, glucose 6-phosphate; GK, glucokinase; HK I, hexokinase I; UDP-Glc, UDP-glucose; GKRP, glucokinase regulatory protein; PGM, phosphoglucomutase; UDPGPP, UDP-glucose pyrophosphorylase; GP, glycogen phosphorylase; G6Pase, glucose-6-phosphatase

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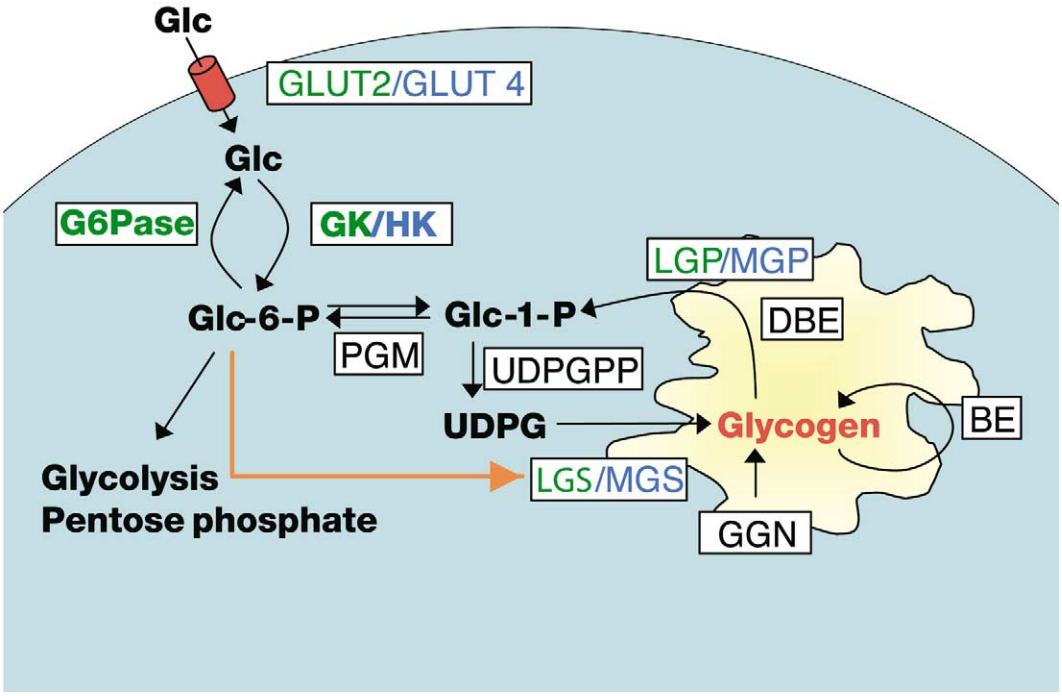

Fig. 1. Schematic representation of glycogen metabolism in liver and muscle. In green are shown the liver proteins and in blue the corresponding muscle isoforms. G6Pase is only expressed in liver and there is no muscle isoform. In addition to the abbreviations used in the text, BE and DBE indicate, respectively, the branching and debranching enzymes and GGN indicates glycogenin.

In muscle, glucose is imported mainly by GLUT-4, a high-affinity and low-capacity glucose transporter. In the absence of insulin GLUT-4 is localized in internal vesicles, which translocate to the plasma membrane in response to the hormone [8]. Thus, in contrast to what occurs in liver, glucose transport in muscle is mainly insulin dependent. On the other hand, muscle does not possess GK but expresses two isoforms of HK [9], I and II, both of which show a low K_m for glucose (in the micromolar range) and are inhibited by low concentrations of Glc-6-P.

In the biochemical pathway towards glycogen synthesis, Glc-6-P is converted successively into Glc-1-P, by phosphoglucomutase (PGM), and then into UPD-glucose, by UDP-glucose pyrophosphorylase (UDPGPP). This last metabolite is the glycosyl donor in the reaction catalyzed by GS. Neither PGM [10] nor UDPGPP [11] has tissue-specific isoforms, and therefore muscle and liver express the same forms of these two enzymes. In contrast, two isoforms of mammalian GS have been described. Most organs express the muscle form, whereas the liver isoenzyme appears to be tissue specific [3]. Although the two forms are 70% identical in the amino acid sequence, the amino- and the carboxyl-terminal, which contain the known phosphorylation sites that regulate the activity of the enzyme, show a much lower degree of homology [12]. The C-terminal region of the enzyme also encompasses a stretch of sequence that is involved in the binding of Glc-6-P [13]. These differences and their dissimilar intracellular distribution [14,15] suggest that muscle and liver GS have distinct capacities in the control of glycogen synthesis in the respective organs (Fig. 2).

Glycogen phosphorylase (GP) catalyzes the key step in glycogen degradation [16], yielding Glc-1-P. GP is regulated by allosteric mechanisms and by phosphorylation of Ser-14 by phosphorylase kinase. The dephosphorylated form (GP *b*) is less active than the phosphorylated form (GP *a*). As with GS, muscle and liver express distinct GP isoforms. Liver GP, unlike the muscle form, is more tightly controlled by phosphorylation than by allosteric regulation [16], and thus not activated by AMP, whereas this compound can overcome the effect of phosphorylation on the activity of the muscle isoenzyme.

3. Activation of liver GS

A fundamental question in hepatic glycogen metabolism is how an increase in blood glucose levels triggers the activation of hepatic GS. Activation occurs through the dephosphoryla-

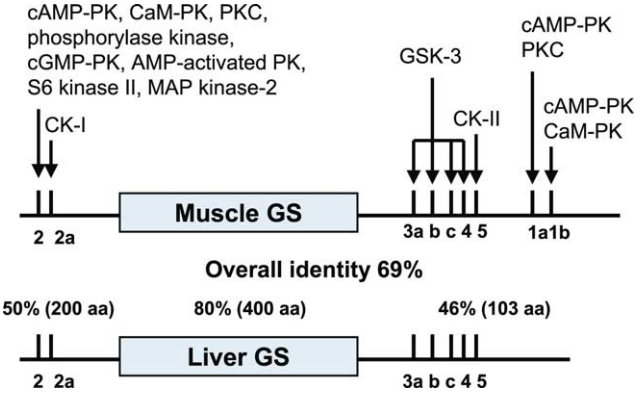

Fig. 2. Comparison of muscle and liver GS primary structures. The central region, which presumably contains the catalytic residues, shows the highest degree of identity between the two isoforms. The known phosphorylation sites at the N-and C-terminal tails, which show a lower degree of identity, are indicated, together with the kinases that are know to phosphorylate these sites in vitro.

tion of the enzyme, which is probably produced by type I protein phosphatases [1]. It was suggested that unmetabolized glucose was indirectly responsible for the activation of GS through a sequential mechanism [17], which involved the prior inactivation of GP. Thus, the binding of glucose to GP *a* would favor its dephosphorylation and inactivation, which in turn would relieve GS phosphatase from the allosteric inhibition caused by GP *a*, but not by GP *b*. Consequently, the dephosphorylation of GP *a* would lead to the dephosphorylation and activation of GS.

However, evidence from our laboratory indicates that glucose must be phosphorylated to promote activation of liver GS. Initially, we showed that the activation of GP was not a prerequisite for the activation of GS. This conclusion was derived from two studies in which the treatment with fructose [18] or lithium chloride [19] of hepatocytes isolated from fasted rats led to the simultaneous activation of GS and GP. Then, we showed that GS activation showed a strong positive correlation with the intracellular levels of Glc-6-P [20]. Later, by incubating isolated hepatocytes with different sugar derivatives, we found that only those hexoses that can give a phosphate ester in C6, such as glucose and 2-deoxyglucose, activate GS [21].

Taken together these results indicate that the activation of GS requires Glc-6-P. The mechanism suggested is that Glc-6-P binds to GS causing the allosteric activation of the enzyme through a conformational rearrangement that simultaneously converts it into a better substrate for protein phosphatases [2]. The action of these phosphatases can then lead to the covalent activation of GS. Therefore Glc-6-P must be considered as both a precursor and a signal molecule to direct glucose incorporation into glycogen (Fig. 3).

Recent studies by our group, using cultured hepatocytes, have shown that Glc-6-P arising from the catalytic action of GK is much more effective in mediating the activation of liver GS than the same metabolite produced by HK I [22,23]. These results indicate that the potency of Glc-6-P for activation of GS is determined by its source. One reasonable explanation for this observation would be that Glc-6-P is compartmentalized in at least two pools in hepatocytes. Liver GS is excluded from the compartment where the Glc-6-P produced by HK I is directed, while it has access to the Glc-6-P pool replenished by the GK-mediated phosphorylation of glucose [24]. This second pool is also accessible to other enzymes and provides a substrate for several metabolic routes. Further evidence along these lines was obtained from the study of hepatoma FTO2B cells, which endogenously express liver GS and HK I, but not GK. These cells do not synthesize glycogen, even in the presence of high concentrations of glucose [25] or when liver GS is overexpressed [24]. However, expression of GK conferred to these cells the ability to synthesize and accumulate glycogen, as well as enhanced glycolysis [24,25]. Overexpression of GK in primary cultured hepatocytes greatly enhanced glycogen accumulation [22]. Furthermore, restoration of normal GK levels in cultured hepatocytes from diabetic rats improved glucose utilization and storage [26].

We have also shown that Glc-6-P arising from gluconeogenesis is as effective in activating hepatic GS as Glc-6-P produced by GK [27]. Therefore, the gluconeogenic pathway and the direct phosphorylation of glucose by GK deliver their

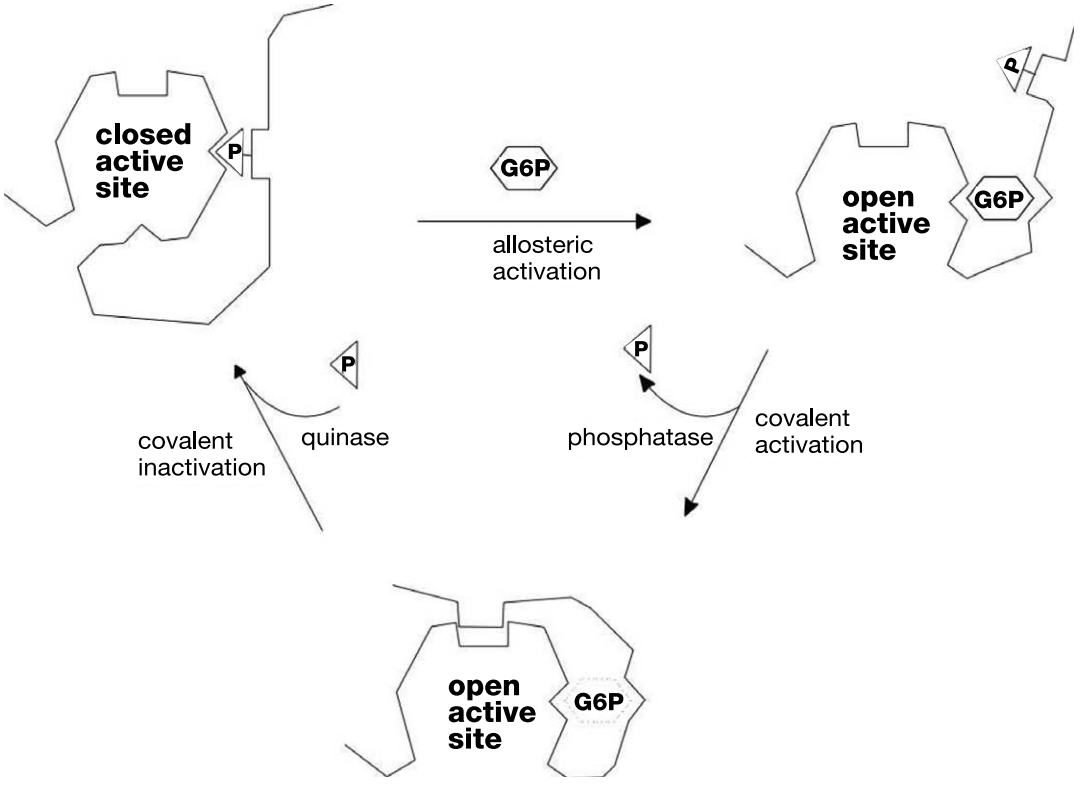

Fig. 3. Schematic representation of the allosteric and covalent activation of GS. Binding of Glc-6-P to phosphorylated inactive GS causes a conformational rearrangement that allosterically activates the enzyme and simultaneously exposes the phosphorylated residues of the protein. Dephosphorylation of these residues produces an active enzyme, which does not need the presence of Glc-6-P to maintain its active conformation.

common product to the same 'general' Glc-6-P pool, which feeds the above-mentioned metabolic processes. On the other hand, Glc-6-P from this pool can also be directed to hydrolysis by glucose-6-phosphatase (G6Pase), because overexpression of the catalytic subunit of this system reduces glycogen deposition and lactate production, while it increases hydrolysis of Glc-6-P [28].

Nevertheless, through the use of GP inhibitors which cause its inactivation by dephosphorylation, it has been shown that under certain conditions GP *a* can play a role in controlling the phosphorylation state of GS [29–31]. These results may be reconciled with the main role of Glc-6-P by assuming that the levels of active GP influence the GS activation state, as long as the intracellular concentration of Glc-6-P remains constant. However, an increase in the Glc-6-P concentration would override the allosteric inhibition of GS phosphatase by GP *a*, thus causing a corresponding increase in the levels of active GS in cultured hepatocytes.

The ability to differentiate between Glc-6-P produced by GK or by HK I is a specific feature of liver GS. The activation state of muscle GS heterologously expressed in FTO2B cells strongly correlated with the intracellular Glc-6-P levels, regardless of the origin of this metabolite [24].

4. Control of glycogen deposition

As mentioned before, it is generally accepted that GS is the enzyme that catalyzes the rate-limiting step in the synthesis of glycogen. The importance of this enzyme in the overall process of glycogen deposition is confirmed by the observation that overexpression of GS in cultured hepatocytes increases glycogen accumulation [32]. This is the consequence of an increase in active GS, induced by endogenous GK activity, since more substrate is available for the Glc-6-P-dependent dephosphorylation of GS catalyzed by phosphatases.

However, when GK is overexpressed, the increase in Glc-6-P results in a higher degree of activation of the endogenous GS, which also leads to the deposition of larger amounts of glycogen. When both enzymes are overexpressed, there is a combination of the two effects. The positive effect of Glc-6-P in glycogen deposition is not due to a 'push' that increases substrate concentration, since UDP-Glc levels are not significantly increased in GK-overexpressing cells. An increase in Glc-6-P produced by GK results in the dephosphorylation of GS molecules, and therefore in an increase in active GS, which in turn 'pulls' substrate into glycogen. These results confirm that the catalytic concentration of 'active' GS (in the I or *a* form) is the key factor in glycogen biosynthesis. When glucose is used as a substrate, this concentration is determined by the levels of both total GS and GK [32].

In metabolic control analysis, the degree of control that a given enzyme exerts over a metabolic pathway is expressed as its control coefficient. The fact that GK overexpression greatly enhances glycogen deposition indicates that GK has a high, positive control coefficient in this process. Its experimental measure afforded a value well over 1.0 [33]. Since the summation theorem dictates that the sum of the control coefficients of all enzymes in a metabolic route must equal 1.0, other proteins of the pathway must possess high negative control coefficients. However, G6Pase, the enzyme that catalyzes the reverse reaction of GK, has a moderately high control coefficient of −0.3 in glycogen deposition. The protein that counterbalances GK is GKRP, which has a negative and high (in absolute value) control coefficient in glycogen synthesis [34]. Thus, the control coefficients of both GK and GKRP in glycogen synthesis are a function of their relative ratio, such that the mechanism comprising these two proteins confers on the hepatocyte a versatile mechanism to adjust glucose phosphorylation far beyond the sensitivity and responsiveness provided by a single sigmoidal enzyme alone.

In summary, hepatic glycogen deposition from glucose is subject to a system of control in which the 'controller', GS, is in turn controlled by GK and GKRP. This system is different from phosphorylation cascades, in which an enzyme directly controls the activity of the next in the pathway. In our system, control is exerted indirectly via the level of Glc-6-P, which 'switches on' GS dephosphorylation, thus leading to its activation.

The control of muscle glycogen synthesis, however, resides in different steps. Measurements from transgenic animals that overexpress a constitutively active form of rabbit muscle GS have shown that, in skeletal muscle, the control of glycogen synthesis is shared between glucose transport and GS [35,36]. In these studies, the authors showed that muscle GS overexpression increased the glycogen content of various types of skeletal fibers. However, the levels of UDP-Glc substantially decreased as a result of the increased GS activity, and the lack of substrate limited glycogen accumulation. HK I, which accounts for a high percentage of total HK activity in skeletal muscle [37], and muscle GS are, respectively, at the beginning and at the end of the glycogen synthesis pathway in muscle. The high affinity of HK I for glucose implies that this sugar is readily converted into Glc-6-P upon entering the cell, and thus the pair HK I–muscle GS is sensitive to the low concentrations of glucose present in the muscle cell. This explains why the control of glycogen deposition in muscle does not reside in the glucose-phosphorylating capacity of the cell but rather in the insulin-stimulated import of the sugar by the GLUT-4 transporter and in GS [35].

As mentioned above, extracellular glucose is in equilibrium with intrahepatic glucose due to the kinetic properties of the hepatic glucose transporter, GLUT-2 [38]. Although GK is the main glucose-phosphorylating activity of the hepatocyte, HK I is also present at considerable levels [39]. When blood glucose is below ~5 mM there is no significant flux through GK because of its high K_m for glucose [39] and the action of its regulatory protein, which further decreases the apparent affinity of GK for glucose in the hepatocyte [40]. Therefore, under these conditions only HK I can phosphorylate glucose, but the Glc-6-P thus produced cannot be diverted toward the synthesis of glycogen since this metabolite does not activate liver GS. Only when the blood sugar concentration increases above a threshold level does GK start to produce Glc-6-P, thus giving the signal that triggers the synthesis of hepatic glycogen. In this case, the control of glycogen synthesis is not exerted by glucose transport but rather by GK and GS [32]. It thus appears that the inability of the Glc-6-P produced by HK I to stimulate the activation of liver GS is one way for the hepatocyte to ensure that hepatic glycogen synthesis is only engaged when needed, which is when blood glucose levels are high.

We conclude that the characteristics of the two pairs of isoenzymes, liver GS/GK and muscle GS/HK, and the rela-

tionships that they establish are tailored to suit specific metabolic roles of the tissues in which they are expressed.

5. Intracellular localization of enzymes

Most of the enzymes that have been described change their intracellular localization in response to glucose, which probably constitutes an additional mechanism of control.

In the absence of the sugar, GK is localized to the nucleus of the hepatocyte, where it is retained by GKRP, but moves into the cytosol when the levels of glucose increase [41,42]. HK I has been shown to reversibly bind to the outer mitochondrial membrane through a hydrophobic N-terminal sequence [43]. This association, which is partly controlled by the intracellular levels of Glc-6-P, plays a role in the regulation of HK I activity in vivo. Low levels of Glc-6-P favor the association with mitochondria and stimulate HK I activity, while high levels have the opposite effect [44].

Muscle GS concentrates in the nucleus at low glucose and translocates to the cytosol, where it adopts a particulate pattern, when the glucose concentration increases [15,45]. In contrast, liver GS presents a cytosolic distribution in the absence of glucose and accumulates at the periphery of the hepatocyte when the concentration of the hexose increases [14,46]. Glc-6-P is responsible for both the activation and translocation of GS [47–49]. It appears that the Glc-6-P-induced change in the allosteric configuration of this enzyme which facilitates its dephosphorylation also triggers the translocation toward the periphery of the cell.

Changes in the intracellular distribution of liver GS and GK triggered by glucose correlate with stimulation of glycogen synthesis [50]. The translocation of these two enzymes suggests a spatial order in the metabolism of hepatic glycogen. In hepatocytes isolated from fasted rats the particles that can give rise to new glycogen synthesis concentrate near the plasma membrane and, initially, new glycogen is synthesized only at the periphery of the hepatocyte. Thereafter, glycogen deposits grow from the periphery towards the interior of the cell forming a crown that becomes thicker as the incubation time with glucose increases. Nevertheless, at least while there is net accumulation of glycogen, the synthesis of the polysaccharide is always active near the plasma membrane. Previously synthesized glycogen moves towards the center of the cell and newly synthesized molecules take its place. As the synthesis of the polysaccharide progresses, glycogen synthesis becomes active at internal sites of the hepatocyte, in addition to the region close to the plasma membrane [51]. GS distribution closely resembles that of glycogen, strongly suggesting that after the initial movement to the cellular cortex and while the deposition of glycogen is active, GS remains bound to its substrate and product (Fig. 4).

Glycogen degradation also seems to take place in an orderly

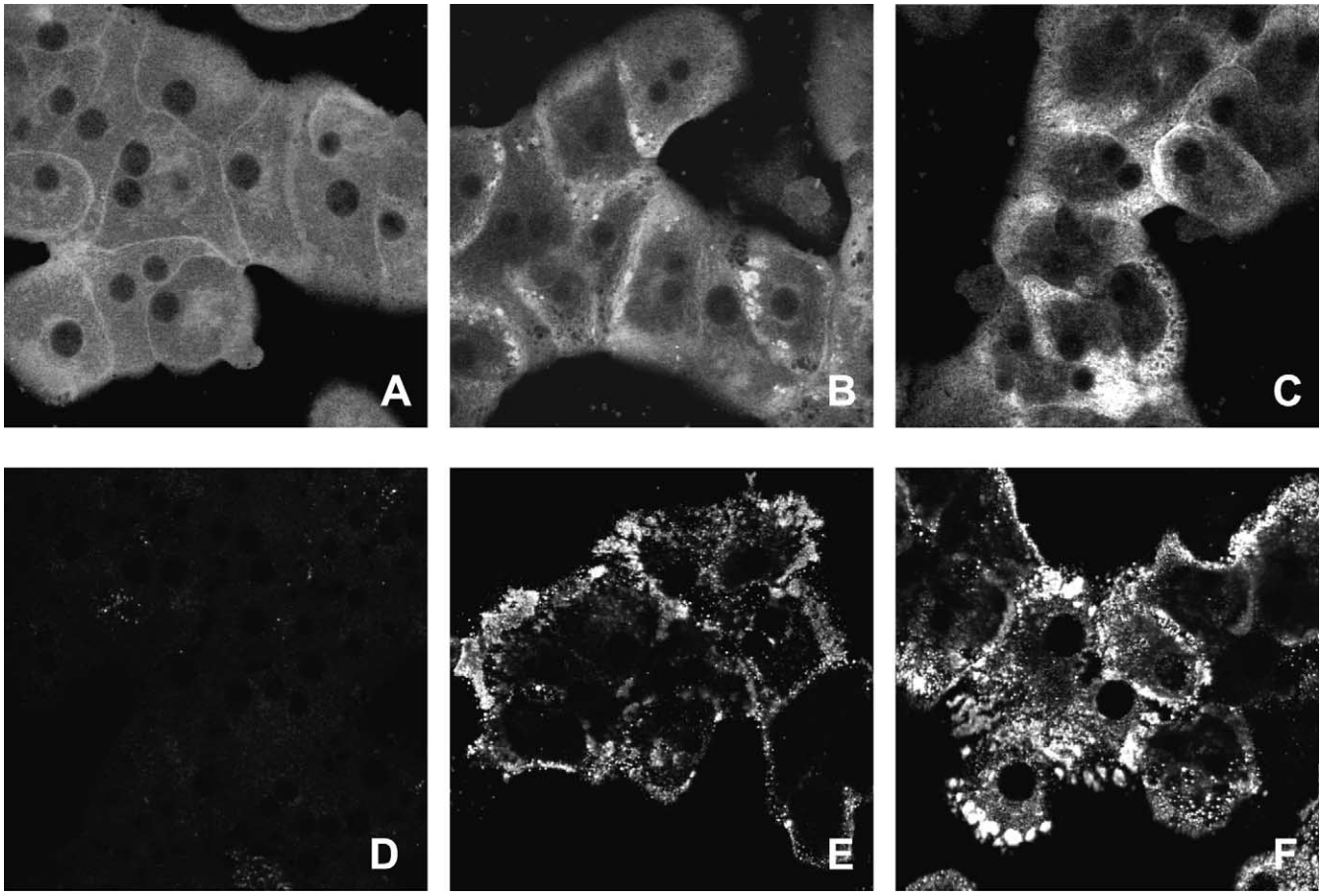

Fig. 4. Intracellular distribution of GS and glycogen in cultured hepatocytes. GS (panels A–C) and glycogen (panels D–F) were detected with specific antibodies in cultured rat hepatocytes, which had been incubated with 30 mM glucose for 0 (panels A and D), 3 (panels B and E) and 6 h (panels C and F). The distribution of GS closely resembles that of glycogen.

fashion. When cultured hepatocytes with full glycogen reserves are depleted of glucose, GS patches gradually become smaller but remain at the cell periphery, suggesting that the remaining undegraded glycogen is also localized there. The GP-mediated phosphorolysis of glycogen might proceed exclusively from the interior to the exterior of the hepatocyte or simultaneously throughout the cytoplasm. However, in the latter case, remaining glycogen particles must move towards the hepatocyte periphery as the degradation of the polysaccharide progresses, in such a way that after an extensive depletion, the very few remaining glycogenogenic particles are located near the plasma membrane. The observation that the molecules of liver glycogen are synthesized in a defined order and degraded in the reverse order, such that glucose units incorporated first are released last during glycogenolysis and vice versa [52], favors the latter hypothesis. This 'ordered' deposition and degradation of glycogen might represent a functional advantage in the metabolism of the polysaccharide or it might simply enable the hepatocyte to store large amounts of glycogen.

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